Mycorrhiza (2023) 33:277–288
https://doi.org/10.1007/s00572-023-01115-7

RESEARCH

Characterization of the arbuscular mycorrhizal fungal community associated with rosewood in threatened Miombo forests

Jean-Marc Kyalamakasa Kaumbu[1,4] · Godar Sene[2,4] · Franck Stefani[3] · Damase P. Khasa[4]

Received: 13 February 2023 / Accepted: 7 June 2023 / Published online: 27 June 2023

Abstract

Understanding the dynamics of arbuscular mycorrhizal fungi (AMF) in response to land use change is important for the restoration of degraded forests. Here, we investigated the AMF community composition in the roots of *Pterocarpus tinctorius* sampled from agricultural and forest fallow soils rich in aluminum and iron. By sequencing the large subunit region of the rRNA gene, we identified a total of 30 operational taxonomic units (OTUs) in 33 root samples. These OTUs belonged to the genera *Rhizophagus*, *Dominikia*, *Glomus*, *Sclerocystis*, and *Scutellospora*. The majority of these OTUs did not closely match any known AMF species. We found that AMF species richness was significantly influenced by soil properties and overall tree density. Acidic soils with high levels of aluminum and iron had a low mean AMF species richness of 3.2. Indicator species analyses revealed several AMF OTUs associated with base saturation (4 OTUs), high aluminum (3 OTUs), and iron (2 OTUs). OTUs positively correlated with acidity (1 OTU), iron, and available phosphorus (2 OTUs) were assigned to the genus *Rhizophagus*, suggesting their tolerance to aluminum and iron. The results highlight the potential of leguminous trees in tropical dry forests as a reservoir of unknown AMF species. The baseline data obtained in this study opens new avenues for future studies, including the use of indigenous AMF-based biofertilizers to implement ecological revegetation strategies and improve land use.

Keywords Arbuscular mycorrhizal fungal community · Land use · Miombo forests · *Pterocarpus tinctorius* Welw. · Soil properties

Introduction

Leguminous trees are important species in tropical dry forests and play a key role in agroforestry (Lebrazi and Fikri-Benbrahim 2022) and soil remediation (Bento et al. 2012) because of their dual symbiotic associations with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (AMF; Glomeromycotina). Legume trees are mainly distributed in the tropics (Castro et al. 2017). In Africa, they are found in a band of tropical seasonal forest known as the Miombo forest which stretches from Angola, the southern Democratic Republic of the Congo (DR Congo), Malawi, parts of Zambia and Zimbabwe, to Mozambique and Tanzania (Timberlake et al. 2010).

In these countries, approximately 100 million people depend on Miombo forests, mainly for agriculture and energy production (Syampungani et al. 2016; Kiruki et al. 2017). These anthropogenic pressures lead to degradation-deforestation and overexploitation of timber, particularly in the case of the rosewood *Pterocarpus tinctorius* (Fabaceae) (Mukenza et al. 2022). *Pterocarpus tinctorius* is widely distributed in Miombo woodlands and is one of the rosewoods that is illegally harvested for its multipurpose uses (Cerutti et al. 2018). This slow-growing evergreen tree is a source of fuelwood, poles, and timber and is used in traditional medicine (Mgumia 2017).

✉ Godar Sene
godar.sene@ucad.edu.sn

1 Ecology, Ecological Restoration and Landscape Research Unit, Agronomy Faculty, University of Lubumbashi, Route Kasapa, Campus Universitaire, Lubumbashi BP 1825, Congo

2 Laboratoire Commun de Microbiologie (LCM) IRD/ISRA/UCAD, Département de Biologie Végétale, Université Cheikh Anta Diop (UCAD), Dakar-Fann BP 5005, Sénégal

3 Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, Ottawa, ON K1A 0C6, Canada

4 Centre d'Étude de La Forêt (CEF) and the Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, QC G1V 0A6 Québec, Canada

The overexploitation of *P. tinctorius* threatens the tropical dry forest ecosystem and reforestation efforts are needed. Production of seedlings with the appropriate root symbionts is required to improve the field survival and sustainable growth of the seedlings (Yonli et al. 2022). However, the AMF community associated with *P. tinctorius* has not been studied. AMF colonize more than 72% of plant species (Brundrett and Tedersoo 2018), facilitating access to water and nutrients, and protecting their hosts from pathogens (Gianinazzi et al. 2010). In return, AMF receive sugars and fatty acids from their host (Luginbuehl et al. 2017; Rich et al. 2021). In degraded soils, AMF increase plant biodiversity, seedling survival, and growth under water or nutrient stress (Asmelash et al. 2016). They alleviate environmental stress and improve soil properties that facilitate plant establishment (van der Heijden et al. 2008). The composition of AMF communities is influenced by the type and intensity of land use (e.g., Sene et al. 2012a, b; Belay et al. 2013; Moora et al. 2014), edaphic properties or soil types (Oehl et al. 2010; Straker et al. 2010), plant species composition, and vegetation type (Sene et al. 2012b; Martínez-García et al. 2015; Rodríguez-Echeverría et al. 2017). These anthropogenic and natural factors act as environmental filters of the AMF community.

In the Zambezian ecoregion, AMF and ectomycorrhizal fungi dominate in degraded and Miombo woodlands (Hogberg and Piearce 1986). However, the AMF diversity is at least twice as high in dry mixed forests and Miombo woodlands (an average of 80 virtual taxa) compared to shrub and grass savannas in Mozambique (fewer than 40 virtual taxa, Rodríguez-Echeverría et al. 2017). In Kenya, AMF spores are abundant in natural forests and artificial plantations compared to cropping systems (Rodríguez-Echeverría et al. 2017).

A comprehensive understanding of the AMF community associated with *P. tinctorius* is important for its successful domestication in the restoration of degraded lands with low inherent AMF abundance and diversity (Asmelash et al. 2016). This study aimed to investigate the community structure of AMF associated with the tropical tree *P. tinctorius* growing in agricultural and forest fallows in relation to host plant composition and soil physicochemical properties. Root samples of *P. tinctorius* were collected from three sites located in the Miombo forest region, Haut-Katanga (DR Congo). Specifically, we expected different AMF communities between agricultural and forest fallows under the influence of (i) tree density and (ii) soil physicochemical variables (e.g., acidity, Al, Fe, and phosphorus). The AMF community was assessed by nested PCR, cloning, and Sanger sequencing of the large subunit (LSU) region of the ribosomal RNA (rRNA) gene.

Materials and methods

Study site

The study was carried out at three sites located in the Haut-Katanga province, south-eastern DRC (Table 1, Fig. Sa, b). At each site, one plot categorized as agricultural fallow (FA) and one plot categorized as forest fallow (FF) were selected. The soil is dominated by haplic ferralsols with a low distribution of plinthic and rhodic ferralsols (FAO 2006; Ngongo et al. 2009). The climate of the Haut-Katanga is defined as a humid subtropical climate and corresponds to the Cwa climate (C = mild temperate, w = dry winter, a = hot summer) according to the Köppen-Geiger classification (Peel et al. 2007). There is a unimodal rainfall pattern consisting of a single rainy season (from November to March), a dry season (from May to September), and transitional months (October and April) (Malaisse 1997).

The abundance of tree and shrub species that were colonized by AMF in the agricultural (FA) and forest (FF) fallows is reported for each site in Table S4. The dominant woody species such as *Albizia* spp., *Baphia bequaertii*, *Pterocarpus* spp. (Table S4), *Combretum collinum*, and *Pterocarpus tinctorius* (Kaumbu, personal observation) in the Miombo forest fallows are mainly colonized by AMF. *Albizia* spp., *B. bequaertii*, and *Pterocarpus* spp. are also found in the agricultural fallows, together with *Diplorhynchus condylocarpon* (Table S4) and are associated with herbaceous species such as *Hyparrhenia variabilis* and *Pteridium aquilinum*. Preliminary results (data not shown) showed that legume species such as members of the genus *Pterocarpus* were the most abundant in all land uses, however, and were more colonized by AMF than other plant species. The Kasenga FA was dominated by *Diplorhynchus condylocarpon*, *Pterocarpus angolensis*, *Combretum collinum*, and *Pterocarpus tinctorius*, while the FF was dominated by *Diplorhynchus condylocarpon* and *Pterocarpus tinctorius*. At the Lubumbashi site, *Albizia adianthifolia*, *Dalbergia boehmii*, *Pterocarpus tinctorius*, and *Syzygium guineense* were the most abundant species in the FA. *Dalbergia boehmii* was reduced in the FF from which *Albizia adianthifolia* and *Syzygium guineense* both were absent. The abundance of *Pterocarpus tinctorius* and *Diplorhynchus condylocarpon* increased in the Lubumbashi FF. The Mulungwishi FA was dominated by *Dalbergia boehmii* and *Pterocarpus tinctorius*. *Pterocarpus tinctorius* also dominated the FF. The other common species were mainly *Diplorhynchus condylocarpon*, *Pericopsis angolensis*, and *Combretum collinum*. All these shrubs and trees are colonized with AMF.

Table 1 Geographical location, land-use of the sampling sites, number of root samples collected, and physico-chemical characteristics of each site

	Kasenga		Lubumbashi		Mulungwishi		*F* value		
	Kipeta	Musangala	Kipopo	Mikembo	Village	Center			
Altitude (m)	978	984	1286	1212	1239	1206			
Longitude (E)	28°32′39″	28°35′36″	27° 24′ 0.2″	27°40′10″	26°33′15″	26°37′46″			
Latitude (S)	10°26′34″	10°27′34″	11° 34′ 22″	11° 28′ 47″	10°44′31″	10°46′33″			
Land-use	FA	FF	FA	FF	FA	FF			
Sample no	5	6	5	5	6	6	Sites	Plots	Sites*plots
pH H_2O	5.33 ± 0.04a	5.27 ± 0.05a	4.78 ± 0.06b	4.74 ± 0.04bc	5.36 ± 0.03a	4.82 ± 0.07b	26.4***	7.99**	8.8**
Acidity (cmol + / kg)	0.35 ± 0.01c	0.39 ± 0.04bc	1.03 ± 0.04a	1.06 ± 0.11a	0.45 ± 0.07b	0.92 ± 0.08a	78.7***	3.12 ns	5.09*
Ca (cmol + / kg)	0.42 ± 0.05a	0.2 ± 0.03ab	0.57 ± 0.12a	0.09 ± 0.01b	0.47 ± 0.12a	0.41 ± 0.05a	8.04**	5.63*	9.9***
K (cmol + / kg)	0.58 ± 0.03a	0.23 ± 0.01 cd	0.38 ± 0.02b	0.21 ± 0.01d	0.26 ± 0.02c	0.38 ± 0.01b	7.39**	21.3***	125.9***
Mg (cmol + / kg)	0.63 ± 0.04ab	0.17 ± 0.04c	0.42 ± 0.06bc	0.12 ± 0.02c	0.91 ± 0.09a	0.46 ± 0.04ab	12.5***	0.03 ns	19.4***
Na (cmol + / kg)	0.24 ± 0.01d	0.11 ± 0.02c	0.11 ± 0.001c	0.03 ± 0.001a	0.03 ± 0.003a	0.06 ± 0.01b	85.1***	0.87 ns	64.6***
C (%)	1.01 ± 0.06ab	0.49 ± 0.02a	2.52 ± 0.06d	1.14 ± 0.08c	1.47 ± 0.02bc	1.49 ± 0.12c	46.6***	0.87 ns	11.4***
Org M (%)	3.2 ± 0.22ab	1.9 ± 0.17a	7.6 ± 0.42d	4.1 ± 0.28bc	4.5 ± 0.42c	5.2 ± 0.36c	44.9***	1.1 ns	17.5***
N (%)	0.05 ± 0.01c	0.03 ± 0.003c	0.15 ± 0.004a	0.08 ± 0.01b	0.09 ± 0.01ab	0.09 ± 0.01ab	39.3***	1.56 ns	8.12**
S (%)	0.01 ± 0.001b	0.01 ± 0.001b	0.02 ± 0.001a	0.01 ± 0.001b	0.012 ± 0.001b	0.013 ± 0.001b	14.7***	0.79 ns	14.33***
CEC (cmol + / kg)	2.62 ± 0.23ab	1.11 ± 0.06c	2.76 ± 0.26ab	1.72 ± 0.17bc	3.17 ± 0.42a	2.53 ± 0.21ab	7.29**	0.21 ns	12.01***
BS (%)	85.37 ± 2.81a	63.63 ± 4.05b	56.7 ± 4.78bc	36.21 ± 6.38c	87.4 ± 4.25a	62.03 ± 5.33b	18.4***	4.31*	11.5***
P_{tot} (mg/kg)	110.1 ± 8.58b	39.81 ± 2.29c	76.5 ± 11.2b	77.94 ± 10.8b	113.4 ± 8.93b	272.8 ± 25.3a	23.8***	24.4***	7.25**
Pbray2 (ppm)	11.1 ± 3.66a	12.9 ± 4.34a	12.37 ± 0.72a	4.49 ± 1.01c	6.05 ± 1.27b	10.11 ± 1.03a	1.2 ns	1.7 ns	9.03***
Al (mg/kg)	1790.7 ± 13.03b	1689.05 ± 18.54a	5304.2 ± 182.1e	2961.6 ± 182.2c	3388.9 ± 251.1 cd	4400.2 ± 172.5de	82.9***	0.82 ns	29.9***
Fe (mg/kg)	1432.8 ± 57.02b	262.05 ± 18.54a	1272.4 ± 70.9b	1619.8 ± 134.3b	2362.3 ± 218.9bc	6132.4 ± 474.3c	55.4***	31.3***	1.87 ns
Sand (%)	54 ± 0.89bc	77.83 ± 0.49a	28.8 ± 0.43d	51.6 ± 0.98bc	43.26 ± 0.6c	56.4 ± 1.37b	39.4***	7.4*	56.1***
Clay (%)	24.6 ± 2.97b	12.67 ± 0.56c	35.5 ± 0.99a	26.17 ± 1.64b	25.5 ± 1.37b	29.67 ± 1.58b	27.7***	1.85 ns	18.58***
CSilt (%)	11 ± 0.57bc	5.83 ± 0.59c	20 ± 0.75a	11.33 ± 1.5bc	15.33 ± 2.13ab	6 ± 1.2c	14.3***	15.5***	16.01***
FSilt (%)	15.4 ± 1.56a	3.67 ± 0.3c	14.33 ± 0.73a	9.33 ± 0.51b	17.83 ± 0.64a	9.17 ± 0.55b	14.4***	2.26 ns	81.3***

Means are followed by standard errors of non-transformed physico-chemical variables. Within rows, the same letters indicate no difference between treatments (i.e., sites and land uses) according to Tukey's HSD tests (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; and ns $p > 0.05$ (not significant))

Sample no. number of samples, *E* east, *S* south, *FA* agricultural fallow, *FF* forest fallow, *pH H_2O* bulk pH in water, *Ca* calcium, *K* potassium, *Mg* magnesium, *Na* sodium, *C* organic carbon, *Org M* organic matter, *N* total nitrogen, *CEC* cation exchange capacity, *S* sulfur, *BS* base saturation, *P_{tot}* total phosphorus, *Pbray2* extractable phosphorus, *Al* total aluminum, *Fe* total iron, *CSilt* coarse silt, *FSilt* fine silt

Sampling procedure and floristic inventory

Rhizosphere soil and root samples were collected from *P. tinctorius* trees in July and August 2016. Five to six trees (Table 1), spaced approximately 100 m apart, were sampled along a transect line. Five grams of fine roots was sampled by selecting two secondary living roots on opposite sides of a tree and excavating the root from the base of the tree until the fine root branching zone was found. Roots were washed in tap water and kept fresh in glycerol-alcohol solution (50%, v/v). At the same site, 200–300 g of rhizospheric soils (immediately surrounding *P. tinctorius* roots) was collected from the upper (0–20 cm) for physico-chemical analyses.

A floristic inventory was carried out in a 10 m radius around each sampled tree to identify the herbaceous and woody species associated with *P. tinctorius*. The number of stems was counted and recorded for each species. Species were identified in the field and unidentified specimens were collected and taken to the botanical laboratory of the Faculty of Agricultural Sciences, University of Lubumbashi, DRC, for further examination.

Soil physico-chemical analyses

The soil was dried at room temperature for 1 week and sieved through a 2 mm mesh. The subsequent physico-chemical analyses were carried out in the soil laboratory of the Faculty of Forestry, Geography and Geomatics of the Université Laval (Québec, QC, Canada). Standard protocols were used for the physico-chemical analyses. Soil texture was determined from the percentage of clay (< 0.002 mm), fine silt (0.002 to 0.02 mm), coarse silt (0.02 to 0.05 mm), and sand (0.05 to 2 mm) fractions by the improved hydrometric method (Bouyoucos 1962). The pH was measured electrometrically in a suspension of 10 g of soil and 20 mL of distilled water.

Soil chemical properties, including pH, extractable cations (calcium, magnesium, potassium, and sodium), cation exchange capacity (CEC), and base saturation, were determined using standard procedures (Bray and Kurtz 1945). Total aluminum, iron, and phosphorus were dissolved according to the manufacturer's instructions (CEM Corporation 2016). Elements were then measured by inductively coupled plasma spectrometry (ICP Agilent 5110 SVDV).

Available phosphorus (Pbray2) was extracted using the two-reagent system (0.03 N NH_4F: 0.1 N HCl) of Bray and Kurtz (1945) and analyzed by flow injection analysis (FIA) using Quikchem method 12–115-01–1-A (Zellweger Analytics, Lachat Instruments Division, Milwaukee, WI, USA). Analyses of total nitrogen and organic carbon were performed according to the Kjeldahl and Walkley–Black methods, respectively. Total nitrogen, carbon, and sulfur were determined by high-temperature combustion and infrared detection in an elemental analyzer (TruMac CNS, LECO Instruments ULC, Mississauga, ON, Canada). The amount of soil organic matter was measured directly as the weight loss during combustion.

DNA extraction, polymerase chain reaction (PCR) amplification, cloning, and sequencing of the large subunit (LSU) rRNA gene

To determine the composition of the AMF community in roots, total genomic DNA was isolated from 50 to 60 mg of root fragments (1 cm in length), ground, and homogenized for 6 min using a SpeedMill PLUS (Analytik Jena AG, Jena, Germany). DNA was extracted using the QIAGEN DNeasy Plant Mini Kit protocol (QIAGEN, Mississauga, ON). The quality and quantity of the DNA was measured using a Nanodrop 1000 spectrophotometer (Thermo Scientific Inc., Wilmington, DE, USA).

The LSU region of the rRNA gene was amplified by targeting the D1 and D2 domains using a nested PCR method (Procter et al. 2014; Crossay et al. 2018). The first-round amplification was performed using the primer set LR1 (5′-GCA TAT CAA TAA GCG GAG GA-3′)/NDL22 (5′-TGG TCC GTG TTT CAA GAC G-3′) (Van Tuinen et al. 1998). The second-round amplification was performed with the AMF specific primer set LR1/FLR4 (5′-TAC GTC AAC ATC CTT AAC GAA-3′) (Gollotte et al. 2003).

The first-round amplification was performed in a final volume of 25 µL containing 16.3 µL of water; 2.5 µL of 10 × buffer; 2.5 µL of T4 gene 32 protein (25 mg/mL); 0.5 µL of deoxy-nucleoside triphosphates (dNTPs, 0.2 mM); 0.5 µL of each primer (25 mM); 0.2 µL of *Taq* polymerase (1 unit/reaction); and 2 µL of genomic DNA. The thermo-cycling conditions were as follows: initial denaturation at 95 °C for 4 min, followed by 35 cycles of 95 °C for 1 min, 56 °C for 1 min, 68 °C for 1 min, and final extension at 68 °C for 10 min.

The first-round PCR products were diluted 1:50 prior to the second-round amplification. The second-round amplification was performed in a final volume of 23 µL, containing 3 µL of DNA; 15.3 µL of water; 2.5 µL of 10 × buffer; 0.5 µL of BSA (bovine serum albumin, 25 mg/mL); 0.5 µL of deoxy-nucleoside triphosphates (dNTPs; 0.2 mM); 0.5 µL of each primer (25 mM); and 0.2 µL of *Taq* platinum (1 unit/reaction). The thermo-cycling conditions were as follows: initial denaturation at 95 °C for 4 min, followed by 30 cycles of 94 °C (45 s), 60 °C (45 s), 72 °C (45 s), and a final extension at 72 °C for 5 min. Amplicons were visualized by electrophoresis on 1% agarose gel in TAE buffer after staining with ethidium bromide. PCRs were performed on an PTC-225 Tetrad Peltier thermal cycler (MJ Research Inc., Waltham, MA).

Second-round PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Ltd. Crawley, UK), and then cloned into the pGEM-T or pGEM-T Easy vector according to the Promega protocol. A minimum of 16 clones per sample were amplified and visualized as previously described for the second-round amplification. Amplicons were sequenced using the Sanger method on the Genomic Analysis Platform at the Institute of Integrative and Systems Biology (IBIS, Université Laval, Québec, QC, Canada). The LSU sequences were deposited in the NCBI GenBank database under accession numbers (available at the time of publication).

Bioinformatic and phylogenetic analyses

The LSU sequences were edited and cleaned using the BioEdit Sequence Alignment Editor v7.2.6.1 (Hall 1999). Sequences were grouped into operational taxonomic units (OTUs), using the 97% similarity threshold in Geneious v9.0.5 (2015, Biomatters, Auckland, New Zealand). A phylogenetic analysis was performed to determine the relationships of the OTUs to known species of AMF. For this, each OTU consensus sequence was identified with the

closest sequences found in the NCBI GenBank database using BLAST (Altschul et al. 1990), in MaarjAM databases (Öpik et al. 2010), and sequences from reference cultures (Krüger et al. 2012). Sequences were aligned using the MAFFT v7 "Auto" algorithm (Katoh and Standley 2013) as implemented in Geneious v9.0.5. A Bayesian phylogenetic tree was inferred using MrBayes v3.2 as implemented in Geneious v9.0.5. A total of 20,000 phylogenetic trees were generated and the consensus tree was calculated by excluding the first 3000 trees.

Alpha and beta diversity analyses

AMF diversity was estimated using the number of OTUs as a proxy for species richness. Arbuscular mycorrhizal community structure was estimated by calculating the frequency of occurrence (FO), relative abundance (RA), importance value (IV), species richness, Shannon–Wiener index, Simpson index, and Pielou evenness index as described in Wang et al. (2019). Briefly, these parameters can be calculated by the following formulas: FO = (number of samples in which the species or genus was observed/total number of samples) × 100%; RA = (number of species or genus/total number) × 100%; IV = (FO + RA)/2. The diversity indices (species richness, Shannon index, Simpson index, and Pielou index) were calculated using the R package *vegan* v2.5–3 (Oksanen et al. 2019) in the R software v3.4.4 (R Core Team 2018). Tree density data were correlated with diversity indices of AMF and edaphic properties to assess the relationships between soil properties, AMF diversity indices, and plant species using the function *rcorr* as implemented in the R package *Hmisc* (Harrell 2018). Correlations were plotted using the R package *corrplot* (Wei and Simko 2017). Normality was checked with the Shapiro–Wilk test while the homogeneity of variance, the independence of residuals, and linearity were assessed with the Goldfeld-Quandt (*gqtest*), Durbin-Watson (*dwtest*), and Rainbow (*raintest*) tests, respectively. These tests were performed using the R package *lmtest* (Zeileis and Hothorn 2002). Rarefaction curves based on the number of samples were calculated using the *specaccum* function as implemented in the R package *vegan*.

The effects of land-use and soil parameters on the AMF community structure were assessed with linear mixed-effects models using the R package *lme4* (Bates et al. 2015). Sites and land-use types were considered as fixed factors and *P. tinctorius* individuals as random factors. Significant differences between sites and their interaction were compared using Tukey's post hoc tests (Hothorn et al. 2008).

The effects of tree density and soil properties on the presence-absence and abundance of indicator OTUs were assessed using canonical correspondence analysis (CCA) and canonical redundancy analysis (RDA), respectively. Significant AM indicator fungal species were identified using the *MRT* and *IndVal* functions as implemented in the R packages *MVPARTwrap* (Ouellette 2011) and *labdsv* (Roberts 2019).

The effect of land-use and soils on the community composition of AMF was assessed using permutational multivariate analysis of variance (PERMANOVA) with the function *adonis2* as implemented in the R package *vegan*. The metric used was Bray–Curtis dissimilarity and 999 permutations were applied to the data. The variation in AMF composition was visualized with non-metric multidimensional scaling (NMDS), using the Bray–Curtis dissimilarity distance metric. NMDS was calculated using the function *metaMDS* as implemented in the R package *vegan*. Overall tree density was projected as an explanatory variable and the influence of the density of *P. tinctorius* was tested with the function *envifit* as implemented in the R package *vegan*.

The overlap of the AMF communities recorded in sites categorized as agricultural fallow (FA) and forest fallow (FF) was visualized with Venn diagrams using the R package *VennDiagram* v1.6.20 (Chen 2018), while the bipartite networks were visualized with the R package *bipartite* v2.15 (Dormann et al. 2008).

Results

Molecular diversity of AMF

Amplification, cloning, and sequencing of 33 *P. tinctorius* field-root samples yielded 355/412 (87% of total) AMF sequences. These sequences were grouped into 30 OTUs, belonging to five genera, two families (Gigasporaceae and Glomeraceae), and two orders (Diversisporales and Glomerales, Fig. S6). Fourteen OTUs were assigned to the genus *Rhizophagus*, five to the clade *Glomus*-1, five to the genus *Dominikia*, five to the genus *Sclerocystis*, and one to the genus *Scutellospora*. Only four OTUs had pairwise similarities greater than 95% with sequences from well identified herbarium cultures. Indeed, OTUs 4, 14, 15, and 19 had pairwise similarities of 95.5%, 99.2%, 97.5%, and 96.4% with *Rhizophagus arabicus* (OTU 4), *Scutellospora erythropa* (OTU 14), *Rhizophagus clarus* (OTU 15) and *Rhizophagus dalpeae* (OTU 19), respectively (Table 2). The three most abundant OTUs may likely represent new species. OTU 1, of which the closest relative was *Rhizophagus neocaledonicus* (93.5% similarity threshold), was the most abundant (RA = 25%) and dominant (FO = 69.7% with significance value of 47.6%). OTU 2 (uncultured *Sclerocystis*, 95.8% similarity) and OTU 3 (uncultured Glomeromycotina, 94.7% similarity) had a relative abundance of 9.3% and 8.45%, respectively, and none of them had a pairwise similarity closely related to a known species (Table 2).

Table 2 Operational taxonomic units (OTUs) of AMF associated with the roots of *P. tinctorius*, ranked according to their total sequence abundance

OTU	OTU abundance										Closest GenBank BLAST			
	Total	KFA	KFF	LFA	LFF	MFA	MFF	FO (%)	RA (%)	IV (%)	AMF species	QC (%)	Identity (%)	Access no
1	91	18	5	24	22	13	9	69.70	25.63	47.67	*Rhizophagus neocaledonicus*	98	93.61	KY362436
2	33	7	1	5	2	6	12	42.42	9.30	25.86	Uncultured *Sclerocystis*	99	95.81	MH504073
3	30	3	1	11	7	3	5	45.45	8.45	26.95	Uncultured Glomeromycotina	99	94.67	KT826604
4	29	1	10	1	3	8	6	39.39	8.17	23.78	*Rhizophagus arabicus*	98	95.45	KF154767
5	22	14	8	0	0	0	0	18.18	6.20	12.19	Uncultured *Sclerocystis*	100	92.88	MH504023
6	21	1	3	5	4	7	1	33.33	5.92	19.62	*Rhizophagus neocaledonicus*	99	93.35	KY362436
7	21	0	5	5	4	6	1	30.30	5.92	18.11	*Rhizophagus neocaledonicus*	97	90.35	KY362438
8	17	1	0	1	2	6	7	30.30	4.79	17.55	Uncultured *Sclerocystis*	97	94.34	MH504023
9	11	4	5	0	0	2	0	21.21	3.10	12.16	Uncultured *Glomus*	99	92.62	HE858410
10	9	0	0	0	2	1	6	18.18	2.54	10.36	*Rhizophagus arabicus*	99	92.68	KF154767
11	7	1	0	0	6	0	0	12.12	1.97	7.05	*Glomus* sp.	98	93.22	KY114652
12	7	0	0	2	0	5	0	6.06	1.97	4.02	*Glomus* OTU 40	99	94.18	HG996315
13	7	0	0	1	2	2	2	12.12	1.97	7.05	Uncultured Glomeromycotina	97	94.64	KJ484691
14	7	1	1	0	5	0	0	9.09	1.97	5.53	*Scutellospora erythropa*	95	99.20	JF816963
15	6	0	3	0	2	0	1	12.12	1.69	6.91	*Rhizophagus clarus*	100	97.51	FM865543
16	6	0	0	0	1	3	2	12.12	1.69	6.91	*Rhizophagus arabicus*	100	92.17	KF154766
17	6	1	1	0	0	4	0	12.12	1.69	6.91	Uncultured *Glomus*	98	95.01	FR873155
18	6	0	0	0	0	6	0	3.03	1.69	2.36	Uncultured *Glomus*	100	99.56	KJ701465
19	3	0	2	0	0	0	1	9.09	0.85	4.97	*Rhizophagus dalpeae*	96	96.40	MN130951
20	3	0	1	0	2	0	0	6.06	0.85	3.45	*Glomus* sp. 2 SL-2017	96	91.67	KP756522
21	3	2	1	0	0	0	0	6.06	0.85	3.45	*Dominika litorea* isolate Dl-8	98	90.98	MG710519
22	2	0	0	1	1	0	0	6.06	0.56	3.31	*Rhizophagus neocaledonicus*	96	90.64	KY362437
23	1	1	0	0	0	0	0	3.03	0.28	1.66	Uncultured Glomeromycotina	99	89.60	HQ128664
24	1	1	0	0	0	0	0	3.03	0.28	1.66	Uncultured *Rhizophagus*	97	93.89	AB935524
25	1	0	0	0	0	1	0	3.03	0.28	1.66	*Glomus* sp.	99	95.27	MK875626
26	1	0	0	0	0	1	0	3.03	0.28	1.66	Uncultured *Glomus*	100	98.97	AB561104
27	1	0	0	0	0	0	1	3.03	0.28	1.66	*Rhizophagus neocaledonicus*	100	87.80	KY362438
28	1	0	0	0	0	1	0	3.03	0.28	1.66	Uncultured *Glomus*	99	97.50	HQ243163
29	1	0	0	0	0	0	1	3.03	0.28	1.66	*Dominikia disticha*	98	91.65	KJ564145
30	1	0	0	0	0	1	0	3.03	0.28	1.66	Uncultured *Glomus*	99	94.81	HQ242918

K Kasenga, *L* Lubumbashi, *M* Mulungwishi, *FA* agricultural fallow, *FF* forest fallow, *FO* frequency of occurrence, *RA* relative abundance, *IV* importance value

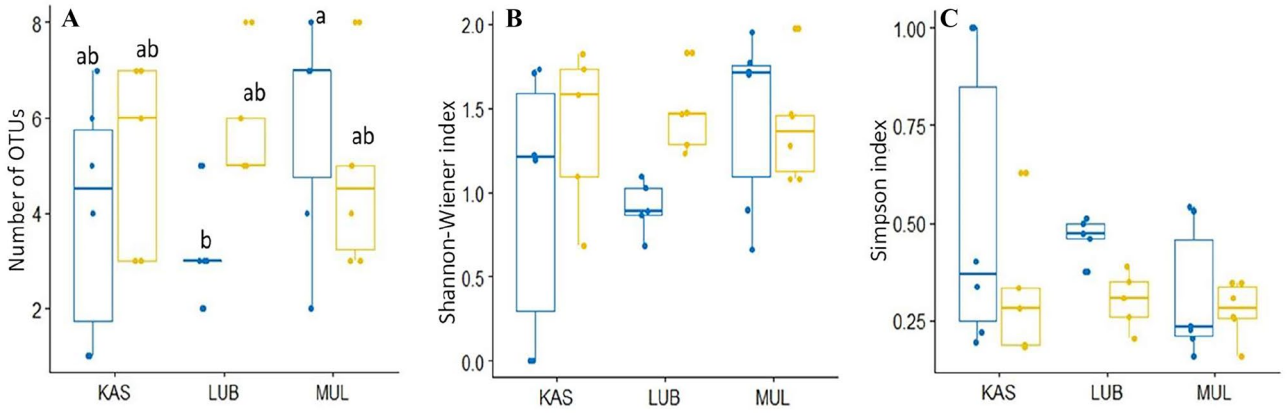

Fig. 1 Diversity of AMF communities observed between agricultural fallow (blue) and forest fallow (yellow) plots for each site (KAS, Kasenga; LUB, Lubumbashi; MUL, Mulungwishi). **A** Number of OTUs (richness), **B** Shannon index, and **C** Simpson index (1-D). Bars topped by the same compact letter display (CLD) indicate no significant differences at $p = 0.05$ by Tukey post hoc tests. Where no CLD are shown, no differences were found

Relationship between AMF diversity and environmental factors

The rarefaction curves showed that most of the AMF diversity associated with *P. tinctorius* roots was captured (Figs. S1 and S5). On average, AMF species richness increased by only one OTU per newly analyzed root sample above the 8th to 10th root sample analyzed.

AMF species richness was the lowest (3 to 5 OTUs, $p < 0.05$) in the roots of *P. tinctorius* growing in the FA plots from Lubumbashi and the highest (2 to 8 OTUs) in the roots of *P. tinctorius* growing in the FA plots from Mulungwishi (Fig. 1A). The linear mixed-effect model shows that only the interaction sites × fallows had a significant effect on species richness ($F_{2,26} = 4.09$, $p = 0.03$). The values of the Shannon and Simpson indices recorded for the root samples from the fallows of three sites were not significantly different at $p = 0.05$ (Fig. 1B, C).

Based on the IndVal analysis, nine OTUs were identified as indicators of pH, base saturation, aluminum, iron, and coarse silt (Fig. 2A). The indicator OTUs were closely related to the species *R. neocaledonicus* (OTUs 1 and 3), *R. arabicus* (OTU 4), *R. irregularis* MUCL43205 (OTU 9 and OTU 10), *S. sinuosa* MD126 (OTU 17), to two unknown species within the genus *Rhizophagus* (OTU 6 and OTU 7) and *Sclerocystis* sp. (OTU 8) (Table S3). Canonical redundancy analysis (RDA) showed that OTUs 9 and 17 (identified as *Glomus* sp.) were significantly related to pH ($p = 0.03$); OTU 3 (unknown Glomeromycotina) was significantly related to acidity ($p = 0.04$) (Fig. S2, *F model* = 1.7; $p = 0.01$; *F first axis* = 6.25; $p = 0.02$).

Canonical correspondence analysis (CCA) showed that the presence of four indicator OTUs 1, 3, 4, and 10 was positively correlated with the tree density of *P. tinctorius*, *Albizia adianthifolia*, *Baphia bequaertii*, *Combretum collinum*, *Strychnos innocua*, and *Syzygium guineense* (Fig. 2B). In contrast, the indicator OTUs 6, 7, and 17 were only positively correlated with the tree density of *Bobgunnia madagascariensis*, *Combretum acutifolium*, *Diplorhynchus condylocarpon*, *Erythrophleum africanum*, *Lannea discolor*, and *Pseudolachnostylis maprouneifolia*. In addition, the tree density of *Anisophyllea boehmii* was positively correlated with the presence of the indicator OTUs 8 and 9.

AMF community structure

PERMANOVA shows a significant effect of sites on the taxonomic composition of AMF (*Pseudo-F* = 1.69, $R^2 = 0.101$, $p = 0.01$). *Rhizophagus* was the most abundant genus in the AMF community (56.6–83.9%), followed by *Sclerocystis* (6.2–41.1%) and *Glomus* (1.8–15.4%). The genera *Rhizophagus*, *Glomus*, and *Sclerocystis* were found in all six plots of the three sites whereas *Dominikia* and *Scutellospora* were absent from the Lubumbashi and Mulungwishi sites, respectively (Fig. 3A). NMDS showed that their abundance was significantly correlated with the tree density of *Albizia antunesiana* ($p < 0.014$), *Anisophyllea boehmii* ($p < 0.04$), and *Dalbergia boehmii* ($p < 0.04$) (Fig. 3B). Tree density was the most important factor determining the composition of the AMF community.

About 50% of the OTUs (16 out of 30) were shared among sites categorized as agricultural fallow (FA) and forest fallow (FF) (Fig. S3). However, the bipartite network showed that five OTUs were present in all six plots (OTUs 1, 3, 4, and 6; genus *Rhizophagus*) and OTU 2 (*Sclerocystis* sp.), representing 57.5% of the relative abundance. Nine

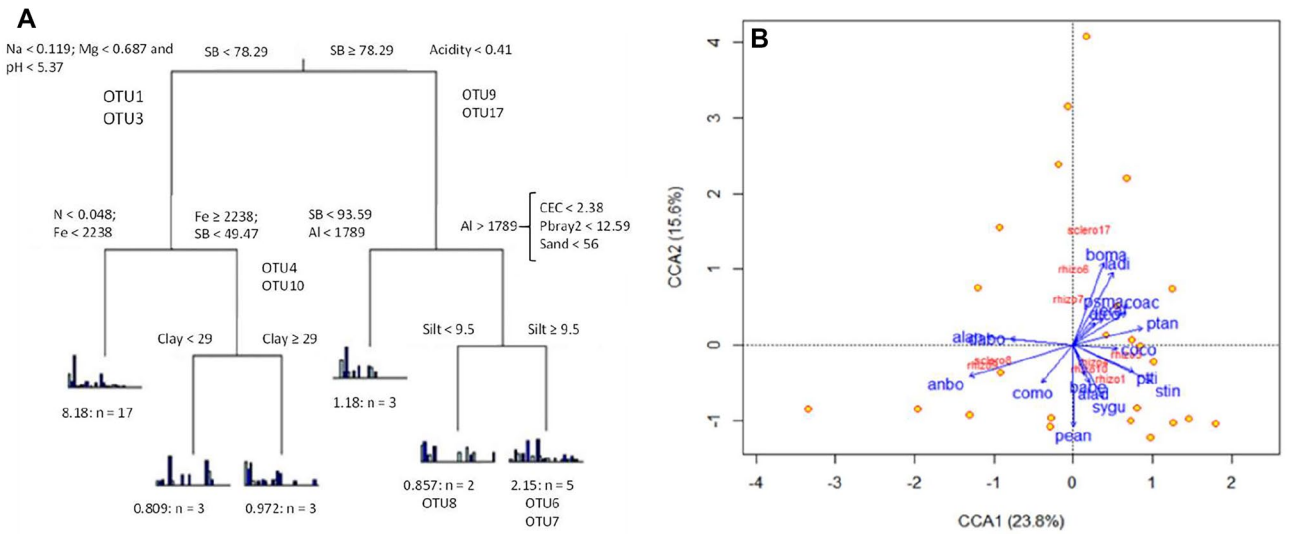

Fig. 2 Relationship between AMF diversity, soil physico-chemical variables, and tree density. **A** Multivariate regression tree showing indicator OTUs of AMFs. Indicator OTUs are shown on either side or at the bottom of the corresponding terminal nodes (leaves), and branches indicate plot separation nodes (n = number of plots per leaf. Error = 0.65; CV error = 1.45; SE = 0.116). **B** Canonical correspondence analysis (CCA) of AMF indicator OTUs with the density of woody tree species coexisting with *P. tinctorius*. OTUs 1, 3, 4, 6, 7, 9, and 10 were assigned to the genus *Rhizophagus* (rhizo) and OTUs 8 and 17 were assigned to the genus *Sclerocystis* (sclero). For CCA, adjusted $R.^2 = 7.6\%$. The vectors on the biplot represent *Albizia adianthifolia* (alad), *A. antunesiana* (alan), *Anisophyllea boehmii* (anbo), *Bobgunnia madagascariensis* (boma), *Baphia bequaertii* (babe), *Combretum acutifolium* (coac), *C. collinum* (coco), *Dalbergia boehmii* (dabo), *Diplorhynchus condylocarpon* (dico), *Erythrophleum africanum* (eraf), *Lannea discolor* (ladi), *Pericopsis angolensis* (pean), *Pseudolachnostylis maprouneifolia* (psma), *Pterocarpus angolensis* (ptan), *P. tinctorius* (ptti), *Strychnos innocua* (stin), and *Syzygium guineense* (sygu)

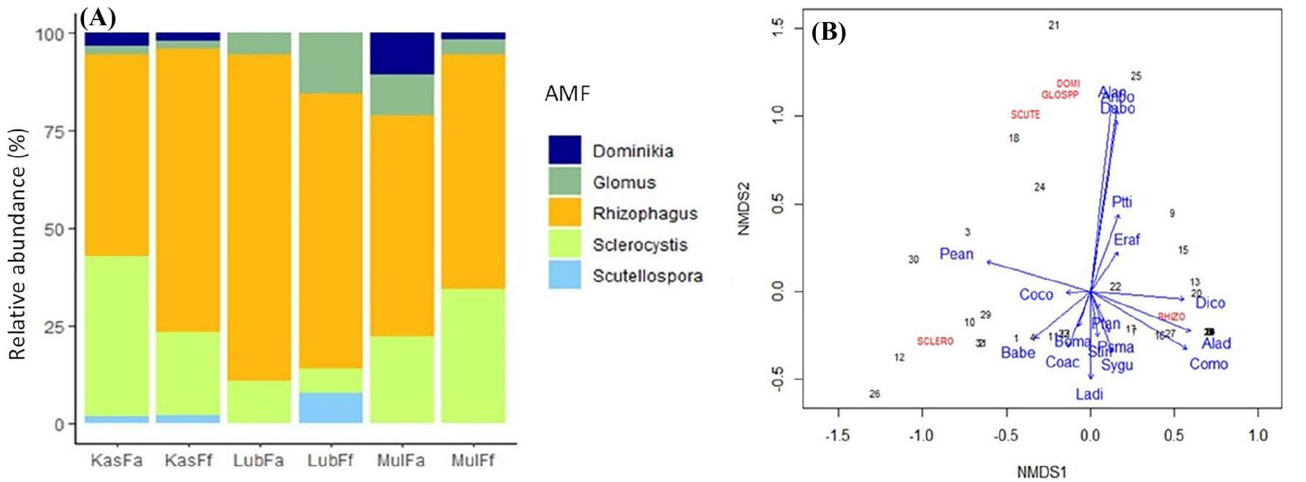

Fig. 3 Community composition of AMF associated with *P. tinctorius* in six plots (KasFa, Kasenga FA; KasFf, Kasenga FF; LubFa, Lubumbashi FA; LubFf, Lubumbashi FF; MulFa, Mulungwishi FA; MulFf, Mulungwishi FF) from agricultural fallow (Fa) and forest fallow (Ff). Kas, Lub, and Mul represent the names of the sites (Kasenga, Lubumbashi, and Mulungwishi, respectively). **A** Relative abundance of AMF genera. **B** Non-metric multidimensional scaling (NMDS) ordination of AMF community composition (GLOSPP, *Glomus* spp.; DOMI, *Dominikia*; RHIZO, *Rhizophagus*; SCLERO, *Sclerocystis*; SCUTE, *Scutellospora*). The plant species are represented by the blue labels (see Fig. 2B and Table S4)

OTUs were detected in only one plot of these three sites, i.e., FA Kasenga (OTUs 23 and 24), FA Mulungwishi (OTUs 18, 25, 26, 28, and 30), and FF Mulungwishi (OTUs 27 and 29).

Discussion

Analysis of the AMF diversity colonizing the roots of the tropical tree *P. tinctorius* growing in agricultural and forest fallow showed a relatively high species richness (30 OTUs). Despite the low throughput of cloning PCR products, the rarefaction curves were close to saturation and the AMF richness recorded in this study is in the range of other studies targeting AMF in root samples using methods based on high-throughput sequencing. For example, 61 amplicon sequence variants were recorded from Illumina sequencing of the small subunit (SSU) region of the rRNA gene in *Tamarix aphylla* root samples (Stefani et al. 2020a). Using pyrosequencing of the SSU region of the rRNA gene, Holste et al. (2016) recorded a total of 22 OTUs in the roots of four tree species growing in southern Costa Rica. However, the number of AMF OTUs reported from root samples can sometimes be an order of magnitude higher. Indeed, a total of 215 OTUs were recorded in the roots of adult trees and seedlings of *Colocasia esculenta* and *Pterocarpus officinalis* in Guadeloupe using pyrosequencing of the SSU region of the rRNA gene (Geoffroy et al. 2017). In their study, those authors showed significant differences in AMF community richness and structure between *P. officinalis* and *C. esculenta*, with the latter being richer in AMF OTUs. In our study, we found that among the plant species associated with *P. tinctorius*, legumes were more colonized by AMF than other plant species (data not shown). As we did not analyze the AMF community associated with the roots of non-legume species, however, the robustness of the comparison remains questionable. In fact, while the AMF recorded in the roots represent the taxa in direct interaction with their host, root analysis provides only a partial view of the structure of the AMF community present below ground.

About 50% of the OTUs recorded in the root samples of *P. tinctorius* were assigned to the genus *Rhizophagus* (Glomeraceae) while only one OTU was assigned to the genus S*cutellospora* (Gigasporaceae). The dominance of taxa assigned to the genus *Rhizophagus* often is observed in roots, and in this sense the genus is aptly named. Hart and Reader (2002) showed that [sic] Glomaceae isolates had high root colonization but low soil colonization and they colonized roots before Acaulosporaceae and Gigasporaceae isolates. In contrast to our study, AMF spores belonging to the families Acaulosporaceae and Gigasporaceae dominate the AMF community in Kenyan ferralsol (Mathimaran et al. 2007).

The majority of the AMF species recorded in the roots of *P. tinctorius* could not be identified to species, suggesting the presence of many undescribed species in the Miombo region. These OTUs representing potential new species were assigned to the genera *Rhizophagus*, *Dominikia*, *Glomus*, and *Sclerocystis*. Only OTUs 15 and 19 (96% similarity to *Rhizophagus clarus*) and OTU14 (97% similarity to *Scutellospora erythropa*) could be related to known species. Consequently, these results show that leguminous trees in tropical dry forests are a potential reservoir of unknown AMF species that need to be fully characterized in order to better assess their role in the restoration of degraded lands.

This is the first time that species of the genera *Dominikia* and *Sclerocystis* have been reported from the Miombo region. In other African regions, the genera *Dominikia* and *Sclerocystis* have been observed in alluvial plains (sand-rich) and degraded sites in secondary succession, respectively. Taxa related to the genus *Sclerocystis* may be globally widespread in the tropics. Members of this AMF genus have been observed in the roots of carob (*Ceratonia siliqua*) from degraded sites in Morocco (Manaut et al. 2015) and in the rhizosphere of karee tree (*Searsia lancea*) from mining sites in South Africa (Spruyt et al. 2014), as well as in three acacia species (*Vachellia abyssinica*, *V. seyal*, and *V. sieberiana*) from a grazing pasture in Ethiopia (Belay et al. 2013). *Sclerocystis* also has been reported from lowland tropical wet forests in Costa Rica and Amazonian Peru (Janos et al. 1995), and in the Asian tropics (Khade and Rodrigues 2003; Tapwal et al. 2013). Sequences assigned to the genus *Dominikia*, although low in abundance, clustered into five OTUs, making the genus the second most species-rich along with the *Glomus-1* clade. *Dominikia disticha* has been reported from marine sands in South Africa (Błaszkowski et al. 2015), which is consistent with our observations where OTUs of this genus were present in root samples from the Lufira (43–56.4% sand, FA Mulungwishi) and Luapula (54–77% sand, FA Kasenga) plains (Table 1).

Results from the CCA and NMDS biplots showed that the community composition of AMF was significantly influenced by the density of *P. tinctorius* trees and other coexisting host-plant species. This affected the abundance of rare genera (*Dominikia* and *Scutellospora*). For example, the AMF genus *Dominikia* and the large shrub flatbean (*Dalbergia boehmii*) were abundant in agriculture fallow (FA) in the Mulungwishi site (less acidic soil and high percentage of base saturation) compared to FA in the Lubumbashi site (acidic soil with high aluminum content). Plants control the AMF community during secondary succession of plant communities, and tree density determines the AMF community and diversity as has been previously reported by Martínez-García et al. (2015) and Sene et al. (2012a, b, 2013). A significant positive correlation also was found between AMF species richness and Pielou's index of woody species. This suggests that an evenly distributed plant community promotes AMF diversity. As

mycorrhizal dependence (Gaidashova et al. 2012) and AMF diversity (Dalpé et al. 2000) differ among plant species, AMF species richness is expected to be higher in sites that are co-dominated by more than two mycotrophic species (Bâ et al. 1996; Lekberg et al. 2013; Mensah et al. 2015; Rodríguez-Echeverría et al. 2017).

The community composition of AMF was significantly influenced by soil properties. Only 5/30 OTUs were observed in the six plots of the three sites. At the genus level, *Rhizophagus* and *Sclerocystis* can be considered generalists (Oehl et al. 2010), as they were present in all plots. OTU 3 is close to *R. neocaledonicus*, which was first described in ultramafic soils of New Caledonia (Crossay et al. 2018). Ultramafic soils, which are rich in Ni and Fe, evolve towards ferralsols under tropical conditions (Echevarria 2017). Other similar AMF sequences have been detected in ferralsols from Kenya (Mathimaran et al. 2007), a country in the same bioclimatic zone as our sampling sites.

In conclusion, the composition of the AMF community did not differ between FA and FF, but it was significantly influenced by the density of *P. tinctorius* trees and other cohabiting woody species. Some OTUs related to the genera *Rhizophagus* and *Sclerocystis* were identified as AM indicators, probably due to their tolerance of aluminum and iron. The abundance of some AMF OTUs was significantly correlated with certain soil properties (acidity, iron, pH, and available phosphorus) and the host tree densities. These relationships suggest co-variation of AMF diversity with host plants, apparently facilitated by soil chemical properties. Furthermore, our results suggest a functional implication for AMF diversity in terms of Al and Fe immobilization and P uptake. The roots of tropical leguminous trees such as *P. tinctorius* are colonized by unknown AMF taxa and trap cultures are required to isolate and characterize these new taxa. The AMF belonging to these taxa could be further isolated and might be used as biofertilizers to improve the restoration of degraded soils.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00572-023-01115-7.

Acknowledgements The authors would like to thank the forest engineers Eric Lowele and Christian Kanunda for their assistance during the floristic inventory, sample collection, and preparation. We thank Stéphane Daigle, research professional in the Centre d'Étude de la Forêt (CEF) for his help with the statistical analyses. Additionally, we thank Marie-Ève Beaulieu for her help with the molecular and bioinformatics analyses. An NSERC Discovery Grant (DPK) is acknowledged. W.F.J. Parsons corrected the English.

Author contributions JMKK and DKP conceived and designed the experiments. JMKK performed all the experiments and analyses. JMKK and GS wrote the first draft of the manuscript. FS co-supervised JMKK and rewrote the subsequent version of the manuscripts with GS. DKP was responsible for supervision and project management, as well as funding and resource acquisition. All authors provided critical input to the drafts and gave final approval for publication.

Funding This study was funded by a doctoral fellowship from the Réseau des Institutions de Formation Forestière et Environnementale d'Afrique Centrale (RIFFEAC), through its project to support the Expanded Program of Training in Natural Resource Management in the Congo Basin (PEFOGRN-BC) funded by the Congo Basin Forest Fund, managed by the African Development Bank. This work also received an NSERC Discovery Grant (DPK).

Availability of data and materials The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

References

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/s0022-2836(05)80360-2

Asmelash F, Bekele T, Birhane E (2016) The potential role of arbuscular mycorrhizal fungi in the restoration of degraded lands. Front Microbiol 7:1095. https://doi.org/10.3389/fmicb.2016.01095

Bâ A, Dalpé Y, Guissou T (1996) Les glomales *d'Acacia holosericea* et *d'Acacia mangium*. Bois & Forêts des tropiques 250:5–18. https://doi.org/10.19182/bft1996.250.a19862

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67. https://doi.org/10.18637/jss.v067.i01

Belay Z, Vestberg M, Assefa F (2013) Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia. Afr J Microbiol Res 7:5503–5515. https://doi.org/10.5897/ajmr2013.6115

Bento RA, Saggin-Júnior OJ, Pitard RM et al (2012) Selection of leguminous trees associated with symbiont microorganisms for phytoremediation of petroleum-contaminated soil. Water Air Soil Pollut 223:5659–5671. https://doi.org/10.1007/s11270-012-1305-3

Błaszkowski J, Chwat G, Góralska A et al (2015) Two new genera, *Dominikia* and *Kamienskia*, and *D. disticha* sp. nov. in Glomeromycota. Nova Hedwigia 100:225–238. https://doi.org/10.1127/nova_hedwigia/2014/0216

Bouyoucos GJ (1962) Hydrometer method improved for making particle size analyses of soils1. Agron J 54:464–465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 59:39–46. https://doi.org/10.1097/00010694-194501000-00006

Brundrett MC, Tedersoo L (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol 220:1108–1115. https://doi.org/10.1111/nph.14976

Castro D, Urzúa J, Rodriguez-Malebran M et al (2017) Woody leguminous trees: new uses for sustainable development of drylands. J Sustain Forest 36:764–786. https://doi.org/10.1080/10549811.2017.1359098

Cerutti PO, Gumbo DJ, Moombe KB et al (2018) Mukula (rosewood) trade between China and Zambia. CIFOR Infobrief 215. https://doi.org/10.17528/cifor/006880

Chen H (2018) VennDiagram: generate high-resolution Venn and Euler plots. Version R package version 1.6.20. https://CRAN.R-project.org/package=VennDiagram

Corporation C (2016) General guidelines to assist with microwave digestion method development

Crossay T, Cilia A, Cavaloc Y et al (2018) Four new species of arbuscular mycorrhizal fungi (Glomeromycota) associated with endemic plants from ultramafic soils of New Caledonia. Mycol Prog 17:729–744. https://doi.org/10.1007/s11557-018-1386-5

Dalpé Y, Diop TA, Plenchette C, Gueye M (2000) Glomales species associated with surface and deep rhizosphere of *Faidherbia albida* in Senegal. Mycorrhiza 10:125–129. https://doi.org/10.1007/s005720000069

Dormann CF, Gruber B, Fruend J (2008) Introducing the bipartite package: analyzing ecological networks. R News 8:8–11

Echevarria G (2017) Agromining: farming for metals, extracting unconventional resources using plants. Mineral Resour Rev 135–156. https://doi.org/10.1007/978-3-319-61899-9_8

FAO (2006) World reference base for soil resources 2006. World soil resources reports No. 103. Food and Agriculture Organization of the United Nations, Rome

Gaidashova S, Nsabimana A, Karamura D et al (2012) Mycorrhizal colonization of major banana genotypes in six East African environments. Agric Ecosyst Environ 157:40–46. https://doi.org/10.1016/j.agee.2012.01.005

Geoffroy A, Sanguin H, Galiana A, Bâ A (2017) Molecular characterization of arbuscular mycorrhizal fungi in an agroforestry system reveals the predominance of *Funneliformis* spp. associated with *Colocasia esculenta* and *Pterocarpus officinalis* adult trees and seedlings. Front Microbiol 8:1426. https://doi.org/10.3389/fmicb.2017.01426

Gianinazzi S, Gollotte A, Binet M-N et al (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530. https://doi.org/10.1007/s00572-010-0333-3

Gollotte A, van Tuinen D, Atkinson D (2003) Diversity of arbuscular mycorrhizal fungi colonizing roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 14:111–117. https://doi.org/10.1007/s00572-003-0244-7

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Harrell FE (2018) Hmisc: Harrell miscellaneous. Version R package version 4.1–1. https://hbiostat.org/R/Hmisc/

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 153:335–344. https://doi.org/10.1046/j.0028-646x.2001.00312.x

Hogberg P, Piearce GD (1986) Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation type and successional patterns. J Ecol 74:775. https://doi.org/10.2307/2260397

Holste EK, Holl KD, Zahawi RA, Kobe RK (2016) Reduced aboveground tree growth associated with higher arbuscular mycorrhizal fungal diversity in tropical forest restoration. Ecol Evol 6:7253–7262. https://doi.org/10.1002/ece3.2487

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346–363. https://doi.org/10.1002/bimj.200810425

Janos DP, Sahley CT, Emmons LH (1995) Rodent dispersal of vesicular-arbuscular mycorrhizal fungi in Amazonian Peru. Ecology 76:1852–1858. https://doi.org/10.2307/1940717

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Khade SW, Rodrigues BF (2003) Occurrence of arbuscular mycorrhizal fungi in tree species from Western Ghats of Goa. India J Trop for Sci 15(2):320–331

Kiruki HM, van der Zanden EH, Gikuma-Njuru P, Verburg PH (2017) The effect of charcoal production and other land uses on diversity, structure and regeneration of woodlands in a semi-arid area in Kenya. Forest Ecol Manag 391:282–295. https://doi.org/10.1016/j.foreco.2017.02.030

Krüger M, Krüger C, Walker C et al (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970–984. https://doi.org/10.1111/j.1469-8137.2011.03962.x

Lebrazi S, Fikri-Benbrahim K (2022) Advances in legumes for sustainable intensification. 461–482. https://doi.org/10.1016/b978-0-323-85797-0.00004-5

Lekberg Y, Gibbons SM, Rosendahl S, Ramsey PW (2013) Severe plant invasions can increase mycorrhizal fungal abundance and diversity. ISME J 7:1424–1433. https://doi.org/10.1038/ismej.2013.41

Luginbuehl LH, Menard GN, Kurup S et al (2017) Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. Sci New York N Y 356:1175–1178. https://doi.org/10.1126/science.aan0081

Malaisse F (1997) Se nourrir en forêt claire africaine. Les Presses Agronomiques de Gembloux, Approche écologique et nutritionnelle

Manaut N, Sanguin H, Ouahmane L et al (2015) Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. Ecol Eng 79:113–119. https://doi.org/10.1016/j.ecoleng.2015.03.007

Martínez-García LB, Richardson SJ, Tylianakis JM et al (2015) Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. New Phytol 205:1565–1576. https://doi.org/10.1111/nph.13226

Mathimaran N, Ruh R, Jama B et al (2007) Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. Agric Ecosyst Environ 119:22–32. https://doi.org/10.1016/j.agee.2006.06.004

Mensah JA, Koch AM, Antunes PM et al (2015) High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. Mycorrhiza 25:533–546. https://doi.org/10.1007/s00572-015-0631-x

Mgumia FH (2017) Traditional uses of miombo woodland tree species in Sikonge District, Tanzania. Int J Nat Resour Ecol Management 2:69. https://doi.org/10.11648/j.ijnrem.20170204.11

Moora M, Davison J, Öpik M et al (2014) Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiol Ecol 90:609–621. https://doi.org/10.1111/1574-6941.12420

Mukenza MM, Muteya HK, Nghonda D-DN et al (2022) Uncontrolled exploitation of *Pterocarpus tinctorius* Welw. and associated landscape dynamics in the Kasenga territory: case of the rural area of Kasomeno (DR Congo). Land 11:1541. https://doi.org/10.3390/land11091541

Ngongo M, Ranst EV, Baert G et al (2009) Guide des sols en République Démocratique du Congo, tome I: étude et gestion. Ecole Technique Salama-Don Bosco

Oehl F, Laczko E, Bogenrieder A et al (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biol Biochem 42:724–738. https://doi.org/10.1016/j.soilbio.2010.01.006

Oksanen J, Blanchet FG, Friendly M et al (2019) vegan: community ecology package. R package version 2.5–6. Version 2.5–6. https://CRAN.R-project.org/package=vegan

Öpik M, Vanatoa A, Vanatoa E et al (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x

Ouellette M-H (2011) MVPARTwrap: additional functionalities for package mvpart. Version 0.1–9. https://cran.r-project.org/src/contrib/Archive/MVPARTwrap/

Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sc 11:1633–1644. https://doi.org/10.5194/hess-11-1633-2007

Procter AC, Ellis JC, Fay PA et al (2014) Fungal community responses to past and future atmospheric CO2 differ by soil type. Appl Environ Microb 80:7364–7377. https://doi.org/10.1128/aem.02083-14

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org.L

Rich MK, Vigneron N, Libourel C et al (2021) Lipid exchanges drove the evolution of mutualism during plant terrestrialization. Science 372:864–868. https://doi.org/10.1126/science.abg0929

Roberts DW (2019) labdsv: ordination and multivariate analysis for ecology. Version 2.0–1

Rodríguez-Echeverría S, Teixeira H, Correia M et al (2017) Arbuscular mycorrhizal fungi communities from tropical Africa reveal strong ecological structure. New Phytol 213:380–390. https://doi.org/10.1111/nph.14122

Sene G, Samba-Mbaye R, Thiao M et al (2012a) The abundance and diversity of legume-nodulating rhizobia and arbuscular mycorrhizal fungal communities in soil samples from deforested and man-made forest systems in a semiarid Sahel region in Senegal. Eur J Soil Biol 52:30–40. https://doi.org/10.1016/j.ejsobi.2012.05.005

Sene G, Thiao M, Manga A et al (2012b) Arbuscular mycorrhizal soil infectivity and spores distribution across plantations of tropical, subtropical and exotic tree species: a case study from the forest reserve of Bandia, Senegal. Afr J Ecol 50:218–232. https://doi.org/10.1111/j.1365-2028.2011.01315.x

Sene G, Thiao M, Samba-Mbaye R et al (2013) The abundance and diversity of legume-nodulating rhizobia in 28-year-old plantations of tropical, subtropical, and exotic tree species: a case study from the forest reserve of Bandia, Senegal. Microbial Ecol 65:128–144. https://doi.org/10.1007/s00248-012-0094-y

Spruyt A, Buck MT, Mia A, Straker CJ (2014) Arbuscular mycorrhiza (AM) status of rehabilitation plants of mine wastes in South Africa and determination of AM fungal diversity by analysis of the small subunit rRNA gene sequences. S Afr J Bot 94:231–237. https://doi.org/10.1016/j.sajb.2014.07.006

Stefani F, Bencherif K, Sabourin S et al (2020a) Taxonomic assignment of arbuscular mycorrhizal fungi in an 18S metagenomic dataset: a case study with saltcedar (*Tamarix aphylla*). Mycorrhiza. https://doi.org/10.1007/s00572-020-00946-y

Straker CJ, Hilditch AJ, Rey MEC (2010) Arbuscular mycorrhizal fungi associated with cassava (*Manihot esculenta* Crantz) in South Africa. S Afr J Bot 76:102–111. https://doi.org/10.1016/j.sajb.2009.09.005

Syampungani S, Geldenhuys CJ, Chirwa PW (2016) Regeneration dynamics of Miombo woodland in response to different anthropogenic disturbances: forest characterisation for sustainable management. Agroforest Syst 90:563–576. https://doi.org/10.1007/s10457-015-9841-7

Tapwal A, Kumar R, Pandey S (2013) Diversity and frequency of macrofungi associated with wet ever green tropical forest in Assam, India. Biodiversitas 14(2):73–78. https://doi.org/10.13057/biodiv/d140204

Timberlake J, Sawadogo ECL, Sawadogo L (2010) Distribution and characteristics of African dry forests and woodlands. In: The dry forests and woodlands of Africa

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Van Tuinen D, Zhao B, Gianinazzi-Pearson V (1998) Mycorrhiza manual. In: Varma A (ed). Springer-Verlag, Berlin Heidelberg New York, pp 387–400

Wang J, Wang GG, Zhang B et al (2019) Arbuscular mycorrhizal fungi associated with tree species in a planted forest of Eastern China. Forests 10:424. https://doi.org/10.3390/f10050424

Wei T, Simko V (2017) R package "corrplot": visualization of a correlation matrix. Version 0.84. https://github.com/taiyun/corrplot

Yonli HH, Sene G, Sanon KB, Dianda M, Khasa DP (2022) *Senegalia senegal* (L.) Britton response to microbial and manure amendments for the rehabilitation of waste rock dumps in the Essakane gold mining site, Burkina Faso. Front Environ Sci 10:803009. https://doi.org/10.3389/fenvs.2022.803009

Zeileis A, Hothorn T (2002) Diagnostic checking in regression relationships. R News 2(3):7–10